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Effects of d-cloprostenol administrations with 7.5 and 11.5-day intervals between administrations on pregnancy rates after artificial insemination in estrous cyclic dairy goats

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ABSTRACT

This study was conducted to evaluate effects of two administrations of d-cloprostenol at different intervals to synchronize the time of estrus and ovulation among estrous cyclic goats. In Experiment 1, 32 does were treated with 30 µg d-cloprostenol at 7.5 (T7.5, $n = 16$) or 11.5-day (T11.5, $n = 16$) intervals. In Experiment 2, the same treatments were administered and there was AI of the does (T7.5, $n = 40$ and T11.5, $n = 38$). In Experiment 1, ultrasonic assessments of ovaries were conducted at the time of the second administration of d-cloprostenol, every 12 h until detection of ovulation, and 7 days after estrous onset to detect the corpora lutea, as well as for pregnancy diagnosis 40 days after AI. In Experiment 1, the estrous response (90.6%, 29/32) was similar ($P > 0.05$) in both groups. Diameter of the largest follicle at the time of administration of the second dose was larger ($P = 0.01$) in the T7.5 than T11.5 group (7.0 compared with 5.7 mm), while the values for ovarian variables were similar ($P > 0.05$). In Experiment 2, the greatest ($P < 0.001$) synchrony in timing of initiation of estrus in does (T7.5 = 83.3% and T11.5 = 50.0%) occurred after the second day (36–48 h). The pregnancy rate tended ($P = 0.0836$) to be greater for does in the T7.5 (71.4%, 40/56) than T11.5 (55.6%, 30/54) group. With use of both protocols, there were acceptable estrous synchronization and pregnancy rates in estrous cyclic dairy goats.

1. Introduction

The number of Brazilian milk goat herds has increased in both production and productivity, especially in the southeast region (Lôbo et al., 2017). Estrous cycle control must be used to optimize the reproductive efficiency of herds. The timing of estrus in estrous

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cyclic goats can be synchronized using protocols with two administrations of prostaglandin F2α (PGF2α) synthetic analogues. These protocols are less onerous and simpler than protocols where progestogen and gonadotropin are used while there can be a desirable synchrony of estrus among does and pregnancy rate in artificially inseminated dairy goats (Maia et al., 2017).

Menchaca and Rubianes (2004) reported that with two administrations of PGF2α at 7-day intervals there can be avoidance of the inhibitory effects of the corpus luteum (CL) on estrous expression and a highly acceptable synchrony of estrus and ovulation rate in ewes. In goats, there has been administration of two doses of PGF2α 10 days apart to synchronize the time of estrus and ovulation among does, but the CL functionality (P4 concentrations) appears to be affected in comparison to when there is a naturally occurring ovulation (Vázquez et al., 2010). Furthermore, there has been a greater than typical incidence of estrous cycles that are of shorter than typical duration for goats treated with PGF2α at 10-day intervals (Fonseca et al., 2012; Esteves et al., 2013). These findings indicate that it is important for evaluation of other intervals such at 7 and 11.5 days for estrous synchronization of does (Maia et al., 2017). By adjusting the time of AI based on the interval to estrus after the last luteolytic treatment, there was a highly acceptable estrous response, estrous synchronization and conception rate (7 days: 85.2% and 11.5 days: 93.5%), whereby there was less variation in the interval to estrus with the 11.5 day interval treatment (Maia et al., 2017).

In sheep, the stage of dominant follicle(s) development - growing or regressing - has been implicated in determining whether there is a relatively earlier or later estrous onset, respectively, after administration of the second dose of a luteolytic agent (Menchaca and Rubianes, 2004). The effects of these physiological variables have not been previously assessed in goats. Considering the average time from the second PGF2α administration to estrous onset, there are two dark periods (*i.e.*, nights) before estrous expression occurs following administration of a second dose of PGF2α if this administration occurs at the end of an afternoon period (Maia et al., 2017).

It was hypothesized that using a 7.5-day protocol with the second d-cloprostenol dose in the afternoon, as with use of the 11.5-day protocol, would lead to the animals having a greater synchrony of estrus. Also, it was expected that with the use of the 7.5-day protocol the second treatment would induce development of a wave in ovarian follicular growth. As a result, the larger follicles would still be in the growing phase, thereby resulting in ovulation from more than one follicle with a similar estrous synchrony occurring compared with that with use of the 11.5 day protocol as previously described (Maia et al., 2017) and with perhaps greater estrous synchrony.

The aim of this study was to investigate the efficacy of estrous synchronization protocols with two d-cloprostenol administrations at intervals of 7.5 or 11.5 days in estrous cyclic dairy goats submitted to a flexible-time AI based on the onset of estrus.

2. Materials and methods

2.1. Ethics and animal care

This study was approved by the Ethics Committee for the Use of Animals of Embrapa Gado de Leite (protocol #3050060218) and was conducted using methods consistent with the principles of the Brazilian Society of Laboratory Animal Science.

2.2. Location and experimental conditions

The study was conducted in two experiments performed during the breeding season from May (estrous synchronization) to July (pregnancy detection). Experiment 1 was performed at the Embrapa experimental campus in Coronel Pacheco (21° 35′S and 43° 15′ W), and Experiment 2 was performed at a commercial dairy goat farm in Ouro Fino (22° 16′ 59″ S and 46° 22′ 08″ W), both in Minas Gerais State, Brazil.

All goats were managed in an intensive system and fed corn silage. A balanced concentrated dietary supplement was provided was given according to their milk production (National Research Council-NRC, 2007). Mineralized salt (Caprinofós® Tortuga, São Paulo, Brazil), and drinking water were available *ad libitum*.

2.3. Experimental animals and treatments

In both experiments, two injections of 30 μg d-cloprostenol (Prolise®, Tecnopec LTDA, São Paulo, Brazil) were administrated by the latero-vulvar route (Fonseca et al., 2017b) at intervals of 7.5 (T7.5) or 11.5 (T11.5) days. There was administration of the first dose on a random day of the estrous cycle. There was initiation of the administrations using the 7.5-day protocol 4 days after there was administration of the first dose with use of the 11.5-day protocol, so there was administration of the second dose for both protocols on the same day and time, in the afternoon.

Experiment 1 was designed to monitor estrus and ovulatory follicular dynamics. In this experiment, 32 estrous cyclic dairy goats (1–4 years old) were equally allocated to two groups based on parity status of does, nulliparous (T7.5, $n = 8$; T11.5, $n = 8$) and pluriparous (T7.5, $n = 8$; T11.5, $n = 8$), body weight (BW) and body condition score [(BCS, 1–5 range; Villaquiran et al., 2007): T7.5 ($n = 16$, BW: 48.2 ± 2.7 kg and BCS: 4.1 ± 0.1) and T11.5 ($n = 16$, BW: 47.6 ± 3.2 kg and BCS: 3.9 ± 0.1)]. At 1 week before starting the treatments, estrous cyclicity was confirmed as a result of detection of a CL in all animals.

Experiment 2 was conducted to determine the conception/pregnancy rates after AI based on synchronized estrous onset. In Experiment 2, 78 pluriparous estrous cyclic dairy goats (2–8 years old) were equally divided into two groups based on age and BCS: T7.5 ($n = 40$, BCS: 3.2 ± 0.1) and T11.5 ($n = 38$, BCS: 3.3 ± 0.1).

2.4. Ultrasonic evaluation

A B-mode and color Doppler trans-rectal ultrasonography assessment of ovaries was conducted on the morning preceding the second d-cloprostenol administration, at the second d-cloprostenol administration, and every 12 h after the second d-cloprostenol administration until ovulation confirmation as well as 7 days after the second d-cloprostenol administration to identify if there were corpora lutea present. All examinations were conducted by the same operator using an ultrasonographic device (Mindray®, M5Vet, Shenzhen, China) with a stiffened, variable frequency (5–8 MHz) linear-array transducer. The goats were maintained in a standing position, fecal pellets were removed (if necessary), and 10 mL of carboxymethylcellulose gel (Carbogel UTL®, Carbogel Indústria e Comércio LTDA, São Paulo, Brazil) was deposited with a syringe in the animal's rectum to lubricate and increase the contact surface. After initial visualization of the urinary bladder, the ovaries and the ovarian structures were located after the rotation of the transducer. For a more precise assessment of the ovarian follicular dynamics, the number, relative position, and size of the follicles ≥ 3 mm were recorded. An ovulation was considered to have occurred on the day when a previously identified dominant follicle or follicles were no longer present. The number of follicles ≥ 5 mm in diameter was recorded (Menchaca et al., 2007). The luteal vascularity percentage was calculated using the formula: (vascularization area / total CL area) x 100 using Adobe Fireworks® and Image J® software packages. A trans-rectal ultrasonic assessment for pregnancy status was conducted approximately 40 days after AI.

2.5. Estrous detection, cervical mucus evaluation, and artificial insemination

After the second administration of d-cloprostenol, evaluations for symptoms of estrus were conducted twice daily (0600 to 0800 h and 1600 to 1800 h) using a “teaser” buck with desirable libido expression based on evaluations made before the experiment was initiated. The AI was performed using a technique conducted by Embrapa staff on a routine basis (Fonseca et al., 2017a) 24, 18 and 10 h after the beginning of estrus for goats with estrous onset at 24 to 36, 36 to 48, and subsequent to 60 h after the second d-cloprostenol administration, respectively (Maia et al., 2017). The cervical mucus evaluation was conducted using procedures that were previously described (Fonseca et al., 2017b), using the 1 to 5 scale as follows: crystalline – 1 (mucus completely translucent); crystalline/striated – 2 (mucus with some opacity but devoid of striation); striated – 3 (evident striation within crystalline areas); striated/caseous – 4 (striation coalescing and no visible translucent areas); and caseous – 5 (mucus appearing as a caseous mass with evident flocculation). The semen used was donated by CapraGene®, the Brazilian Breeding Plan for dairy goats progeny testing (Facó et al., 2011) with 100 to 120 million viable sperm per 0.25 straw before freezing and with at least 40% progressive linear motility (0% to 100% variation) and a vigor 3 score (speed of viable sperms cells; 0 to 5 variation) after thawing in a water bath at 35 °C for 30 s. Buck semen was always thawed in paired samples from each buck sequentially, allowing equal use for paired thawing samples in both groups (T7.5 and T11.5).

2.6. Statistical analysis

All data were initially compared between treatments (T7.5 and T11.5). In Experiment 1, there was an additional statistical analysis conducted to compare the values for reproductive variables between females that had ovulations before 66 h (early ovulating) and from 78 to 90 h (late ovulating) subsequent to the time of the second d-cloprostenol administration as previously described by Murtaza et al. (2019).

Values for continuous variables were reported as the mean ± SEM, and categorical variables were reported in terms of frequency and percentage within the groups. All continuous variables were assessed for normality using the Lilliefors test and variances were evaluated using the Cochran and Bartlett tests. For the continuous variables considered to have a normal distribution, a one-way analysis of variance was conducted and the means were compared using an F-test. The categorical variables were compared using the exact Fisher test or chi-square test, depending on the number of observations. A Pearson correlation was also used to study the associations of dependence between the continuous variables. The probability that was considered to be significant was $P < 0.05$ and a tendency was considered to exist if the probability value was greater than $P > 0.05$ and less than $P < 0.10$. The statistical analysis was performed using SAEG 9.0 software (Ribeiro Júnior, 2001).

3. Results

3.1. Experiment 1

After the second d-cloprostenol administration, the overall estrous response rate was 90.6% (29/32). There was the greatest synchrony in timing of estrous onset (75%) in both groups from 48 to 72 h after the second d-cloprostenol administration. All data regarding follicular dynamics and ovulation are presented in Table 1. One doe in the T7.5 group had the shortest interval to estrus after the second d-cloprostenol administration (12 h). The range in time to ovulation after the second administration of d-cloprostenol was the same for both groups (24 to 90 h).

The number of follicles ≥ 5 mm at the time of the second d-cloprostenol administration, number of ovulations, and total corpora lutea detected were similar for both groups ($P > 0.05$). The number of follicles ≥ 5 mm at the time of the second d-cloprostenol administration was positively correlated with the number of ovulations ($r = 0.54$; $P = 0.01$) and was also positively correlated with total number of CL formed ($r = 0.50$; $P = 0.01$). The number of ovulations was also positively correlated with total number of CL formed ($r = 0.60$; $P = 0.001$). There was a negative correlation between the interval to ovulation and number of ovulations ($r =$

Table 1
Data (mean ± SEM or %) for reproductive variables of estrous cyclic dairy goats administered two 30 μg doses of d-cloprostenol at 7.5 or 11.5-day intervals for estrous synchronization.

Variables	7.5 days	11.5 days	*P* value
Animals (*n*)	16	16	–
Follicles < 5 mm at second d-cloprostenol administration (*n*)*	1.7 ± 0.4	2.4 ± 0.9	n.s.
Follicles ≥ 5 mm at second d-cloprostenol administration (*n*)*	2.9 ± 0.3	2.3 ± 0.2	0.0746
Diameter of largest follicle at second d-cloprostenol administration (mm)	7.0 ± 0.4	5.6 ± 0.3	0.0117
Estrus response (%)	93.8 (15/16)	87.5 (14/16)	n.s.
Interval to estrus (h)**	44.8 ± 3.6	48.9 ± 1.5	n.s.
Interval to ovulation (h)**	65.6 ± 4.3	71.6 ± 4.5	n.s.
Interval from estrus to ovulation (h)	22.7 ± 3.1	26.3 ± 2.5	n.s.
Diameter of largest ovulatory follicle (mm)	7.6 ± 0.2	7.4 ± 0.4	n.s.
Diameter of second largest ovulatory follicle (mm)	6.2 ± 0.2	6.2 ± 0.3	n.s.
Average diameter of ovulatory follicles (mm)	6.6 ± 0.4	6.4 ± 0.2	n.s.
Number of ovulations (*n*)	2.6 ± 0.2	2.4 ± 0.2	n.s.
Total corpora lutea formed (*n*)	2.2 ± 0.2	2.3 ± 0.2	n.s.
Corpora lutea total area including cavity (mm^2)	15.5 ± 2.7	20.7 ± 2.3	n.s.
Corpora lutea total area excluding cavity (mm^2)	14.2 ± 2.6	18.1 ± 2.4	n.s
Luteal vascularity (%)	47.9 ± 8.6	48.9 ± 1.5	n.s.
Conception (%)	53.3 (8/15)	35.7 (5/14)	n.s.
Pregnancy (%)	50.0 (8/16)	31.3 (5/16)	n.s.

* Follicle data were recorded considering both ovaries.
** hours after second d-cloprostenol administration; () Number of animals.

—0.37; $P < 0.05$).

The percentages of does having ovulations before 66 h (early ovulating) from the time of the second d-cloprostenol administration were 61.5% (8/13) and 38.5% (5/13), while those having ovulations from 78 to 90 h were 43.7% (7/16) and 56.3% (9/16) for the T7.5 and T11.5 groups, respectively ($P > 0.05$). Interval to estrus after the second administration of d-cloprostenol tended ($P = 0.052$) to be shorter for does having relatively shorter periods (42.5 ± 3.5 h) as compared with those having relatively longer periods (50.2 ± 2.0 h) before the time when ovulations occurred. The intervals from the second cloprostenol administration to ovulation (54.0 ± 4.0 and 80.2 ± 1.2 h) and from estrous onset to ovulation and from estrus to ovulation (16.4 ± 3.0 and 30.0 ± 1.5 h) were shorter ($P = 0.0001$), respectively, in early than in late ovulating does.

In 55.2% (16/29) of goats, the ovulations occurred from both ovaries, while in 31.0% (9/29) and 13.8% (4/29) ovulations occurred from either the left or right ovary, respectively.

3.2. Experiment 2

As depicted in Fig. 1, by 60 h after the second d-cloprostenol administration, all goats had expressed estrous behavior. The onset of estrus occurred mainly at night, being detected in the morning (*i.e.*, 36 and 60 h) in T7.5 (75%, 27/36) and T11.5 (78.1%, 25/32). Two goats from both groups initiated estrus after the first day, while in the greatest percentage of does in estrus (T7.5 = 83.3%, 30/36; T11.5 = 50.0%, 16/32; $P < 0.001$) was after the second day (36 to 48 h), followed by the third day (T7.5 = 11.1%, 4/36; T11.5 = 43.7%, 14/32).

The values for reproductive variables of estrous induction and AI are included in Table 2. The interval to estrus was shorter ($P = 0.0022$), while the interval from estrus onset to AI was longer ($P = 0.0016$) in T7.5 than in T11.5 groups. The conception rate as associated with the interval to estrus, and consequently to the time of AI after estrous onset, was similar (Table 3).

There was crystalline mucus in two goats, one for each treatment with a 100.0% conception rate in both groups. Crystalline/

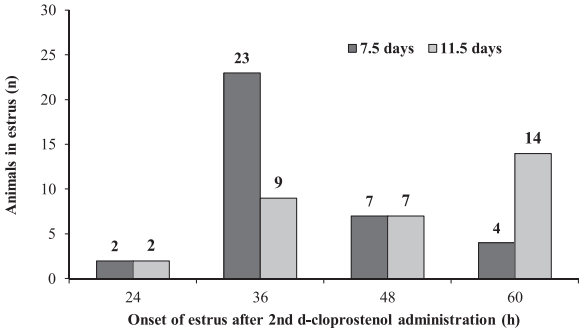

Fig. 1. Number of goats in Experiment 2 based on the interval to estrus onset after hormonal synchronization of timing of estrous onset with two doses of 30 μg d-cloprostenol (PGF2α) at intervals of 7.5 and 11.5 days.

Table 2
Values (mean ± SEM or %) for reproductive variables of dairy goats submitted to estrous synchronization protocols using two doses of 30 μg d-cloprostenol at 7.5 or 11.5-day intervals and inseminated according to the time of estrous onset.

Variables	7.5 days	11.5 days	*P* value
Animals *(n)*	40	38	–
Estrous response (%)	90.0 (36/40)	84.2 (32/38)	n.s.
Interval to estrus (h)*	40.1 ± 1.5	48.4 ± 2.1	0.0022
Interval to artificial insemination (h)*	63.6 ± 0.7	65.9 ± 1.0	0.0610
Interval from estrus onset to artificial insemination (h)	23.5 ± 1.2	17.6 ± 1.4	0.0016
Cervical mucus at artificial insemination (1-5)	3.6 ± 0.1	3.2 ± 0.1	n.s.
Depth of semen deposition (0-5)	4.5 ± 0.2	4.8 ± 0.1	n.s.
Conception (%)	88.9 (32/36)	78.1 (25/32)	n.s.
Pregnancy (%)	80.0 (32/40)	65.8 (25/38)	n.s.

* Hours after second d-cloprostenol administration.

Table 3
Conception rate (%)as affected by the time of estrous onset after a second d-cloprostenol administration in dairy goats submitted to a protocol where there was administration of two doses of 30 μg d-cloprostenol at 7.5 or 11.5-day intervals and with there being artificial insemination (AI) based on the time of estrous onset.

Variables	7.5 days	11.5 days	Total
Animals (*n*)	36	32	68
24 (AI 24 after estrus onset)	100.0 (2/2)	100.0 (2/2)	100.0 (4/4)
36 (AI 24 after estrus onset)	87.0 (20/23)	100.0 (9/9)	90.6 (29/32)
48 (AI 18 after estrus onset)	85.7 (6/7)	71.4 (5/7)	78.6 (11/14)
60 (AI 10 after estrus onset)	100.0 (4/4)	64.3 (9/14)	72.2 (13/18)
Total	88.9 (32/36)	78.1 (25/32)	83.8 (57/68)

() Number of animals. $P > 0.05$.

striated mucus was detected in four goats with resultant conception rates for does of 100.0% (1/1) for the T7.5 and 66.7% (2/3) for the T11.5 group. Striated mucus detection was associated with conception rates of 90.0% (9/10) for the T7.5 and 87.5% (14/16) for the T11.5 group. Striated/caseous mucus detection was associated with conception rates of 87.5% (21/24) for the T7.5 and 66.7% (8/12) for the T11.5 group. Intrauterine insemination resulted in conception rates of 93.3% (28/30) for the T7.5 and 81.5% (22/27) for the T11.5 group ($P > 0.05$).

Overall, considering both experiments, the pregnancy rate tended ($P = 0.0836$) to be greater for the T7.5 (71.4%, 40/56) than the T11.5 (55.6%, 30/54) group.

4. Discussion

The two administrations of d-cloprostenol at 7.5 and 11.5-day intervals were equally effective for synchronizing the time of estrus among estrous cyclic does. Overall, the pregnancy rate tended to be greater for the T7.5 (71.4%) than the T11.5 (55.6%) group. Pregnancy with use of the T7.5 protocol was slightly greater than the 52.5% reported after laparoscopic insemination in ewes treated with two doses of PGF2α 7 days apart (Vilarino et al., 2017). It is important to highlight that in Experiment 1, the pregnancy rate was less than anticipated based on results in previous studies which may have been due to the stress caused by handling the animals for the ultrasonic exams (Dobson et al., 2012; Maia et al., 2017).

The diameter of the largest follicle at the time of the second d-cloprostenol administration was on average 1.4 mm larger in T7.5 than T11.5 group. Also, the number of follicles ≥ 5 mm diameter tended to be greater in the T7.5 (2.9) than in T11.5 (2.3) group. These findings may have resulted from the shorter intervals to estrus, AI, and from estrous onset to AI in the T7.5 compared with the T11.5 group when a larger number of animals was used (Experiment 2). Menchaca and Rubianes (2004) reported that if the second PGF2α was administered during the growing stage of the larger follicles, the onset of estrus was earlier compared with animals where the dominant follicles were regressing. This could explain the differences observed in Experiment 2 for the interval to estrus and AI. Follicular diameters at the time of the second administration of d-cloprostenol were similar for animals with relatively shorter or longer durations of time to ovulation; however, it is possible that there was a difference in the status (growing or regressing) of the largest follicles in the two groups. It is suggested that in animals that had ovulations before 66 h (early ovulating) after the second administration of d-cloprostenol, the largest follicles present at the time of administration of the second dose were growing and that growth of these follicles continued after luteolysis when the inhibitory effect of progesterone on LH secretion ceased as a resulted of the induced luteolysis. The preovulatory follicle may grow to a diameter of an ovulatory follicle earlier in contrast to animals that have large follicle(s) in the regression phase and in which there needs to be development of a new wave of follicular development before ovulation occurs (late ovulating). It, therefore, is consistent, from a physiological perspective, that there were ovulations in a shorter time interval after the second administration of d-cloprostenol with use of the 7.5 as compared with the 11.5-day protocol for estrous synchronization.

To the best of our knowledge, this is the first time that there has been identification of correlations among the number of follicles ≥ 5 mm at the time of the second d-cloprostenol administration, number of ovulations, and total number of CL formed. The correlations among values for these variables indicate that transrectal ultrasonic data, in part, can be used to predict the success of an estrous synchronization protocol as a whole, including some key points for assisted reproductive technologies such as AI or recipient animal preparation/synchrony for embryo transfer. Nevertheless, it is proposed that ultrasonic technologies can be used to indicate the dominant follicle development status (growing or atretic) at the time of administration of the second dose of d-cloprostenol. In addition, de Castro et al. (1999) reported that dominant follicles from the first wave of ovarian follicular development during an estrous cycle had a greater diameter and duration when compared to the dominant follicles developing in subsequent waves of follicular development of an estrous cycle. Ginther and Al-Mamun (2009) reported that PGF2α administered 10 days after ovulation resulted in an increased ovulation rate in mares. This finding is probably resulted from a lesser progesterone profile at the beginning of the estrous cycle, providing a more favorable endocrine milieu for subordinate follicles to overcome the dominance effects of the largest follicles or establishment of co-dominance. This could be the reason why more follicles ≥ 5 mm diameter were observed in does of the T7.5 than T11.5 group.

The diameter of the largest follicle at the second d-cloprostenol administration was not correlated with the intervals to estrus or ovulation; however, as previously emphasized in this manuscript, the developmental and/or viability status of the largest follicles could determine whether there will be a relatively earlier or later onset of estrus. This could be the factor responsible for the extent of synchronization in the time of initiation of estrus among animals. Most animals in Experiment 1 expressed symptoms of estrus between 48 and 72 h after the second d-cloprostenol administration, while in Experiment 2 the does were in estrus earlier after the second administration of d-cloprostenol with a greater synchrony in timing of estrous onset (*i.e.*, before 60 h after the second dose). In the T7.5 group, there was a greater synchrony in the timing of estrous onset (36–48 h after the second dose of d-cloprostenol), which would allow the labor cost to be decreased with use of this protocol compared with most other protocols in a commercial system.

The interval to ovulation was not affected by the time-period between the two d-cloprostenol administrations to synchronize time of initiation of estrus among does (˜ 68.6 h after the second administration). Menchaca and Rubianes (2004) reported that there was a highly synchronized timing of ovulation at about 60 h after the administration of a second dose of PGF2α (7-day interval) to ewes when imposing an estrous synchronization protocol. In the present study, there was a negative correlation between the interval to ovulation and number of ovulations. Although not assessed in the present study, it is likely that there is an association with larger numbers of ovulatory follicles and estrogen concentrations in blood, allowing the threshold concentration of this steroid necessary to induce a pre-ovulatory surge release of LH in a shorter time period after the second administration of d-cloprostenol. As a consequence of this greater estrogen milieu, final follicular maturation and ovulation could also occur in a shorter time period after this administration.

Recently, Murtaza et al. (2019) reported that does ovulating in a relatively shorter timeframe after induction of luteolysis had (1) larger follicles and (2) a smaller CL diameter at the time of PGF2α administration compared with those ovulating later (5.4 ± 0.2 compared with 4.3 ± 0.2 mm and 10 ± 0.6 compared with 11.8 ± 0.3 mm, respectively). In the present study, there was a tendency for greater number of larger follicles in T7.5 than T11.5 group. Nevertheless, the ovulatory response, CL total area, and percentage of vascularization of luteal tissue were similar when both protocols were used for estrous synchronization. Thus, the greater determinant for the shorter period of time to estrous onset observed for does in the T7.5 group was related to the diameter of the largest follicles at the time of the second cloprostenol administration.

Interestingly, there was a slightly lesser number of CL that developed in both groups as compared with the number of ovulations that were detected in these groups. It is possible that there was a partial or premature regression of CL [*i.e.*, normal and abnormal (avascular and pale)] that were present in the same doe, as reported in does where there was an estrous synchronization protocol imposed (Souza-Fabjan et al., 2013), and that these abnormal CL were not identified using ultrasonography.

In the current study, the AI time was defined using the procedures that were previously described for goats (Maia et al., 2017), and this was established based on the optimal characteristics of the cervical mucus (striated or striated-caseous) when AI was performed using frozen-thawed semen (Fonseca et al., 2017b). The pregnancy rates observed in the present study, as well as in a previous study (Maia et al., 2017), indicate there are repeatable results with highly acceptable pregnancy rates expected when there is AI of goats using this methodology.

5. Conclusion

Two cloprostenol administrations at either 7.5 or 11.5-day intervals were very effective in synchronizing the time of initiation of estrus and ovulation in dairy goats. With use of the 7.5-day protocol, there was greater synchrony in the time of onset of estrus from 36 to 48 h after the administration of the second dose of d-cloprostenol and there was a tendency for greater pregnancy rates when compared with use of the T11.5 day protocol. Based on estrous onset results and the AI strategy, the use of both protocols resulted in more than 90% of goats in estrus for insemination on the same day and with adequate cervical mucus characteristics indicating the hormonal milieu was optimal for reproductive tract secretions indicative of estrus.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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